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Effect of Adding Different Levels of Raw Powdered Mulberry and Artichoke Leaves, and Their Combination, on Liver Enzymes and Certain Oxidative Stress Markers in Laying Hens Exposed to Heat Stress

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ABSTRACT

Objective: *This study aimed to investigate the biological effects of dietary supplementation with mulberry and artichoke leaves on mitigating the negative impacts of heat stress in laying hens.* ***Method:*** *A total of 144 Lohmann Brown laying hens were randomly assigned to six dietary treatments (24 hens per treatment, three replicates of eight hens each). The treatments consisted of a basal diet supplemented with artichoke leaf powder, mulberry leaf powder, or their combination at inclusion levels of 0, 1, 1.5, 1, 1.5, and 1.5+1.5 g/kg of feed, respectively. The trial lasted four months, during which blood samples were collected biweekly from each replicate, and monthly averages were analyzed to evaluate liver enzyme activities (AST, ALT, ALP) and oxidative stress markers (MDA, SOD).* ***Results:*** *The findings revealed that dietary supplementation with mulberry and artichoke leaves, individually or in combination, significantly alleviated the adverse effects of heat stress, as reflected by improved oxidative status and more favorable liver enzyme activity.* ***Novelty:*** *This study highlights the functional potential of natural plant-based additives, such as mulberry and artichoke leaves, as sustainable nutritional strategies to enhance resilience and maintain performance of laying hens under heat stress conditions.*

INTRODUCTION

Heat stress is one of the major environmental factors that negatively affect the health, behavior, and productivity of poultry, especially under conditions of extreme temperature elevation [1]. As homeothermic animals, birds maintain a constant body temperature and are highly sensitive to thermal fluctuations, which can lead to disturbances in general physiological functions such as feed intake, growth rate, and overall health deterioration [2]. Therefore, understanding the impacts of heat stress on poultry is essential for developing effective strategies and interventions to mitigate its effects. One such strategy involves the use of dietary supplements, including mulberry and artichoke [3].

The scientific name of mulberry is *Morus alba L.*, commonly known worldwide as mulberry [4], [5], [6]. It is a fast-growing plant that adapts well to high temperatures and belongs to the Moraceae family, which includes two genera: *Morus* (mulberry) and *Ficus*, Mulberry leaves are rich in flavonoids, which are natural antioxidants, including anthocyanins and phenolic compounds, as well as high levels of vitamins C and E and carotenoids [7] Global studies have demonstrated that the bioactive compounds in mulberry play a significant role in protecting against bacterial infections and various diseases [8].

Artichoke (*Cynara scolymus*), a member of the Asteraceae (daisy) family, contains a wide range of biologically active compounds that contribute to its health-promoting effects as a dietary supplement and antioxidant [8]. Accordingly, this study aimed to evaluate the efficacy of raw powdered mulberry and artichoke leaves as dietary supplements in the feed of laying hens exposed to heat stress, by assessing their impact on liver enzyme activity and antioxidant status.

RESEARCH METHOD

This study was conducted to investigate the effect of supplementing artichoke leaf powder, mulberry leaf powder, and a mixture of both to the diets of laying hens subjected to heat stress at the onset of their production phase, focusing on their physiological performance.

A total of 144 laying hens, 23 weeks of age, were randomly allocated into six treatment groups. All experimental birds were fed a unified basal diet supplemented with artichoke powder, mulberry powder, and their mixture at concentrations of 0, 1, 1.5, 1, 1.5, and 1.5 + 1.5 g/kg feed for each respective treatment. The raw powder for each treatment was mixed with a weekly quantity of feed to ensure homogeneity of the added substance with the calculated feed amount for each treatment. The experimental birds were fed a laying hen diet containing 17.75% crude protein. The feed provided per hen ranged from 120 to 130 g/day, while water was supplied ***ad libitum***. Table 1 details the composition of the experimental diet.

The lighting regimen during the study period exposed the birds to 16 hours of light and 8 hours of darkness per day. All birds were subjected to the same temperature conditions for equal durations (an average of 15 minutes every 6 hours). Accurate thermometers were strategically placed within the poultry house (three per house: beginning, middle, and end) to measure temperature. Temperatures were recorded in a dedicated log every 6 hours, resulting in four readings per day, and the average temperature was calculated weekly throughout the study.

Tabel 1. Feed Composition and Chemical Analysis.

Feed Ingredients:

Ingredients	Percentage (%)
Yellow Corn	36.0
Wheat	28.5
Soybean Meal (44% Crude Protein)	16.0
Protein Concentrate	10.0
Limestone	7.7
Sunflower Oil	1.5
Salt	0.3
Total	100

Chemical Analysis of the Diet:

Parameter	Value
Crude Protein (%)	17.75
Metabolizable Energy (kcal/kg feed)	2759
Energy-to-Protein Ratio	155
Lysine (%)	0.86
Methionine (%)	0.41
Methionine + Cysteine (%)	0.68
Calcium (%)	3.06
Phosphorus (%)	0.44

The chemical composition of the diet was determined according to the feedstuff analysis guidelines outlined in the National Research Council report. The protein concentrate used in the formulation was produced by Provimi Company, originating from Jordan.

At the end of each experimental period, blood samples were collected via the brachial wing vein. Two birds were randomly selected from each replicate for each sampling. The area around the vein was cleaned of feathers, disinfected, and wiped with a cotton swab before venipuncture was performed using a 5 mL × 23-gauge plastic syringe. The blood collected from each bird was placed into tubes containing the anticoagulant EDTA for plasma separation. Samples were centrifuged at 3000 rpm to obtain plasma, which was then stored in special glass tubes at –20°C until further biochemical analysis. The activity of the liver enzymes **AST** and **ALT** was determined according to the method described by [9], using commercial assay kits provided by Randox (Spain). The activity of **ALP** was measured following the method of [10], using a diagnostic kit supplied by Linar (Spain). The experiment was conducted using a Completely Randomized Design (**CRD**). Statistical analysis of the data was performed using the Statistical Analysis System software and significant differences among treatment means were compared using Duncan's Multiple Range Test [11].

RESULT AND DISCUSSION

Results

The results presented in Table 2 demonstrate the effects of dietary supplementation with mulberry and artichoke leaf powders, as well as their synergistic combination, on liver enzyme levels and antioxidant status in heat-stressed laying hens at different age stages. From the table, it is evident that during the first experimental period, the overall concentration of the enzyme alkaline phosphatase (**ALP**) was not significantly affected by the dietary treatments. However, during the second experimental period, **ALP** levels showed a significant increase ($P \leq 0.05$) in the groups receiving the dietary supplements T5, T4, T6, and T3 compared to both the T2 treatment group and the control group (T1). In the third period, the increase in **ALP** concentrations was significant in the T4, T5, and T6 groups compared to the remaining treatments.

Similarly, during the fourth experimental period, treatments T6, T4, and T5 exhibited a consistent and significant (P≤0.05) elevation in **ALP** levels compared to the other experimental groups. concerning the **AST** enzyme, the data from the same table indicate that dietary supplementation had no significant effect on **AST** levels during the first and third periods. However, a significant decrease (P≤0.05) in **AST** concentrations was observed in the T5, T4, and T6 treatment groups during the second and fourth periods, compared to the remaining experimental treatments, as for the **ALT** enzyme, no significant effect (P>0.05) was observed due to dietary supplementation during the first and third experimental periods. However, treatments T5 and T4 showed a significant decrease (P≤0.05) in **ALT** concentrations compared to the other treatment groups. This decreasing trend was similarly observed in AST enzyme levels, where treatments T5, T6, and T4, respectively, demonstrated reduced concentrations, concerning oxidative stress markers, the enzyme **malondialdehyde** (**MDA**) showed a highly significant reduction (P≤0.01) in its concentration in favor of treatments T6 and T5 across all experimental periods, compared to the other treatments, For the enzyme **superoxide dismutase** (**SOD**), dietary supplementation had no significant effect on its levels during the first period. However, during the second, third, and fourth periods, treatments T6, T5, T4, and T3 recorded a highly significant increase (P≤0.01) in **SOD** concentrations compared to the remaining two treatment groups.

Table 2. Effect of Raw Artichoke Powder, Mulberry Powder, and Their Synergistic Combination on Liver Enzyme Activity in the Blood Plasma of Heat-Stressed Laying Hens (Mean ± Standard Error).

Traits Period/Tret.		ALP Enzyme (U/I)	AST Enzyme (U/I)	ALT Enzyme (U/I)	MDA Enzyme (U/I)	SOD Enzyme (U/I)
Period1	T1	28.12±2.33	113.12±0.96	10.87±1.01	^{a}2.84±1.02	2.61±0.08
	T2	28.22±3.01	125.14±1.01	10.93±2.13	^{a}3.66±2.70	2.63±0.92
	T3	33.00±2.00	89.33±1.22	12.47±3.02	^{a}2.96±2.33	2.66±0.66
	T4	31.44±1.14	78.80±2.14	10.63±2.44	^{b}2.44±1.01	2.60±0.78
	T5	32.25±2.55	81.14±1.55	10.83±2.36	^{b}2.38±2.02	2.69±0.51
	T6	33.78±5.26	77.90±2.22	10.47±2.11	^{c}2.20±1.66	2.73±0.37
Sig.		**N.S**	**N.S**	**N.S**	**0.01**	**N.S**
Period2	T1	^{b}33.14±1.14	^{a}122.10±2.23	^{a}12.13±1.11	^{a}2.88±1.06	^{a}2.68±0.07
	T2	^{b}33.81±4.25	^{b}104.33±3.32	^{a}11.93±2.55	^{a}2.74±0.93	^{b}2.51±1.05
	T3	^{ab}35.94±6.52	^{ab}98.66±3.12	^{a}11.93±2.14	^{b}2.63±1.05	^{b}2.57±0.66
	T4	^{a}36.14±3.44	^{b}93.13±2.01	^{b}9.20±2.40	^{b}2.60±0.87	^{a}2.65±1.13
	T5	^{a}36.14±4.75	^{c}90.07±2.04	^{c}7.55±1.44	^{c}2.28±1.44	^{a}2.70±0.93
	T6	^{a}38.13±7.02	^{ab}95.44±2.55	^{b}10.33±1.01	^{c}2.30±1.20	^{a}2.80±1.02
Sig.		**0.05**	**0.05**	**0.05**	**0.01**	**0.01**
Period3	T1	^{b}29.14±6.33	130.29±3.12	10.33±2.92	^{a}3.55±1.89	^{b}3.18±1.12
	T2	^{b}31.66±1.30	122.47±3.57	10.88±3.32	^{a}3.40±1.22	^{b}3.28±1.44
	T3	^{b}33.44±3.00	120.66±3.65	10.66±2.08	^{a}3.16±1.40	^{b}3.25±1.22

Traits Period/Tret.		ALP Enzyme (U/I)	AST Enzyme (U/I)	ALT Enzyme (U/I)	MDA Enzyme (U/I)	SOD Enzyme (U/I)
	T4	a36.14±2.14	113.44±3.54	9.81±2.04	b2.48±1.20	a3.61±1.08
	T5	a35.70±4.11	117.14±2.01	9.32±2.38	b2.61±1.88	a3.91±1.11
	T6	a35.15±4.33	111.40±2.31	9.01±2.35	b2.33±1.66	a3.90±1.05
Sig.		**0.05**	**N.S**	**N.S**	**0.01**	**0.01**
Period4	T1	c30.80±1.01	a93.14±1.11	a10.33±1.84	a3.91±0.98	b2.28±0.10
	T2	b33.52±1.12	a88.46±3.01	a10.22±1.37	a3.78±1.05	b2.97±0.14
	T3	b33.18±2.23	ab83.30±3.44	b9.44±2.63	a3.55±0.65	a3.63±0.11
	T4	a37.48±2.55	b80.14±2.04	b9.22±2.14	a3.48±1.13	a3.71±0.22
	T5	a36.47±1.14	bc77.55±2.11	c8.75±2.04	b2.98±1.03	a3.95±0.55
	T6	a38.22±3.06	c63.70±3.00	c9.08±3.66	b2.18±1.66	a3.91±0.41
Sig.		**0.01**	**0.05**	**0.05**	**0.01**	**0.05**

*Different letters within the same column indicate significant differences among the experimental treatments for each study period. The treatments T1, T2, T3, T4, and T5 received a control diet supplemented with raw powders of mulberry leaves, artichoke leaves, or their synergistic combination at the following inclusion levels: (0, 1 g/kg feed of mulberry leaf powder, 1.5 g/kg feed of mulberry leaf powder, 1 g/kg feed of artichoke leaf powder, 1.5 g/kg feed of artichoke leaf powder, and 1.5 g mulberry leaf powder + 1.5 g artichoke leaf powder/kg feed). (**) Indicates highly significant differences among experimental treatments at P≤0.01. (*) Indicates significant differences among experimental treatments at P≤0.05. N.S indicates no significant difference among the experimental treatments.

Discussion

Stress in poultry typically occurs when birds are exposed to environmental changes that disrupt their physiological homeostasis, thereby triggering adaptive responses aimed at restoring internal balance. In the present study, dietary supplementation appeared to play a clear role in mitigating the severity of heat stress [12], [13]. It is well-established that heat stress induces oxidative processes; thus, the bioactive compounds present in mulberry and artichoke—such as flavonoids, cynarin, dietary fibers, inulin, and various phenolic compounds—are believed to exert protective effects by reducing hepatic toxin levels [14], thereby enhancing liver health. This protective role is reflected in the significant reductions observed in the levels of liver enzymes and antioxidant activity markers under investigation. On one hand, these findings suggest improved liver function; on the other, they indicate a mitigation of the known adverse effects of heat stress, which typically include elevated liver enzyme activity and increased antioxidant response due to cellular and hepatic damage. In contrast, the current study showed stable biochemical markers within the physiological range, which may be attributed to the biological effects of the plant-derived compounds

[15]. In **conclusion**, the findings of this study indicate that dietary supplementation with raw mulberry and artichoke leaf powders significantly alleviated oxidative damage induced by heat stress at the cellular level, as evidenced by the modulation of liver enzyme activity and antioxidant defenses.

CONCLUSION

Fundamental Finding : This study demonstrated that dietary supplementation with raw powdered mulberry and artichoke leaves, either individually or in combination, effectively mitigated the negative impacts of heat stress in laying hens, as evidenced by improved oxidative status and favorable modulation of liver enzyme activity. **Implication :** These results suggest that incorporating mulberry and artichoke leaves into poultry diets offers a promising natural and sustainable nutritional strategy to enhance resilience, liver function, and overall health performance under thermal stress conditions, which is highly relevant for poultry production in hot climates. **Limitation :** However, the study was limited to a single poultry breed and focused primarily on biochemical markers without assessing broader production outcomes such as egg quality, feed efficiency, or long-term economic viability. **Future Research :** Further studies should investigate dose optimization, explore synergistic interactions with other functional feed additives, and evaluate the effects on productivity and welfare across diverse poultry genotypes and varying environmental stressors to validate and expand the applicability of these findings.

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